Minireview

Stem cell therapies in the treatment of diabetic retinopathy and keratopathy

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Abstract

Nonproliferative diabetic retinopathy (DR) is characterized by multiple degenerative changes that could be potentially corrected by stem cell therapies. Most studies so far have attempted to alleviate typical abnormalities of early retinopathy, including vascular hyperpermeability, capillary closure and pericyte dropout. Success was reported with adult stem cells (vascular progenitors or adipose stem cells), as well as induced pluripotent stem cells from cord blood. The cells were able to associate with damaged vessels in both pericyte and endothelial lining positions in models of DR and ischemia-reperfusion. In some diabetic models, functional amelioration of vasculature and electroretinograms was noted. Another approach for endogenous progenitor cell therapy is to normalize dysfunctional diabetic bone marrow and residing endothelial progenitors using NO donors, PPAR-δ and -γ agonists, or inhibition of TGF-β. A potentially important strategy would be to reduce neuropathy by stem cell inoculations, either naïve (e.g., paracrine-acting adipose stem cells) or secreting specific neuroprotectants, such as ciliary neurotrophic factor or brain-derived neurotrophic factor that showed benefit in amyotrophic lateral sclerosis and Parkinson's disease. Recent advances in stem cell therapies for diabetic retinal microangiopathy may form the basis of first clinical trials in the near future. Additionally, stem cell therapies may prove beneficial for diabetic corneal disease (diabetic keratopathy) with pronounced epithelial stem cell dysfunction.

Keywords: Diabetic retinopathy, stem cell, neuroprotection, endothelial progenitor cell, diabetic keratopathy

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Introduction

Diabetic retinopathy (DR) has been usually considered as a vascular disease or, particularly, an endotheliopathy manifested by ischemia-induced pathologic alterations in the retinal microvasculature. In recent years, however, it has become more obvious that diabetes-associated neurodegenerative changes take place before endothelial alterations, suggesting that diabetic retinopathy should be regarded as a neurovascular degenerative disease.

A continuous hyperglycemic condition leads to dysfunction and loss of endothelial cells (EC), pericytes and vascular smooth muscle cells, causing hypoxia. This is characteristic of both type I (insulin-dependent) and type II (non-insulin-dependent) diabetes. Specifically, pericyte and EC ability for self-renewal is impaired in diabetes, and their turnover potential is finally depleted,[1,2] after which acellular capillaries become non-perfused, and adjacent retina turns hypoxic. This hypoxic environment upregulates vascular endothelial growth factor (VEGF), which contributes to elevated vascular permeability[3,4] that can result in diabetic macular edema and, eventually, in loss of visual function.[5] Neurodegeneration, which includes neuronal apoptosis and glial cell reactivity, occurs in diabetes before the alterations of ECs are observed.[6–8] In fact, persistent hyperglycemia during early retinopathy can lead to ganglion cell depletion[7] and consequently, to changes in the retinal electrical activity prior to a noticeable endotheliopathy.[9] Neuroretinal hypoxia stimulates the translocation of hypoxia-inducible factor-1α (HIF-1α),[10] which induces the expression of hypoxia-regulated cytokines and growth factors, such as VEGF. VEGF-driven increased proliferation of ECs initially causes intraretinal microvascular abnormalities (IRMA) in areas lacking functional capillaries[11] and may also be responsible for the development of microaneurysms in which ECs proliferate in the absence of pericytes that normally keep ECs dormant. Non-proliferative DR (NPDR) is manifested by capillary non-perfusion, leakage, microaneurysms and IRMAs (Figure 1). EC proliferation and migration from vessels can eventually result in the development of preretinal neovascularization characteristic

of proliferative DR (PDR). More than 3.5% of type I diabetic patients lose vision as a consequence of the disease,[12] which makes DR one of the leading causes of blindness in the world. At present, there are few effective treatments of DR focused on prevention, including tight glycemic and cholesterol control that may maintain sustained protection from disease progression.[13–16] When the disease progresses to sight-threatening macular edema or PDR, laser photocoagulation can help to regress disease by destroying peripheral retina and reducing oxygen demand.[17,18] However, photocoagulation may bring about complications resulting in deteriorating visual acuity, retinal thickening and field loss.[19]

To date, no treatment has yet been developed to support regeneration of the damaged retinal vasculature as a result of long-term hyperglycemia. Cell-based therapies may be a feasible option for both preventing neurovascular damage and promoting regeneration of damaged retina, as evidenced by recent studies with several types of stem cells (Figure 1). Novel approaches developed recently (for reviews see literatures[20–23]) to treat early and moderate DR are mainly based on the ability of mesenchymal stem cells (MSC) to produce neuroprotective and neurotrophic factors, and the potential of endothelial progenitor cells (EPC) to repair vasculature, or the ability of adipose stromal cells (ASC) to accomplish both of the above functions.

Mesenchymal stem cells and their role in diabetes

These are multipotent stromal cells usually isolated from bone marrow, although they can be found in most tissues.[24] *In vitro* cultured MSCs are known to express such surface markers as CD105, CD44, CD90, CD166, CD54 and stromal antigen 1[25] but lack surface markers that are characteristic for hematopoietic cells (CD45, CD11a and CD14).[26] MSCs have recently become possible candidates for use in disease treatment and tissue replacement due to several factors. These include relatively simple donor biopsies that can be expanded *in vitro* and administered intravenously, allowing an autologous treatment. Also, MSCs secrete neuroprotective growth factors such as fibroblast growth factor-2 (FGF-2) and ciliary neurotrophic factor (CNTF),[27] and they proved to be safe in human trials so far.

The ability of MSCs to maintain and restore the neural retina damaged in degenerative diseases was demonstrated

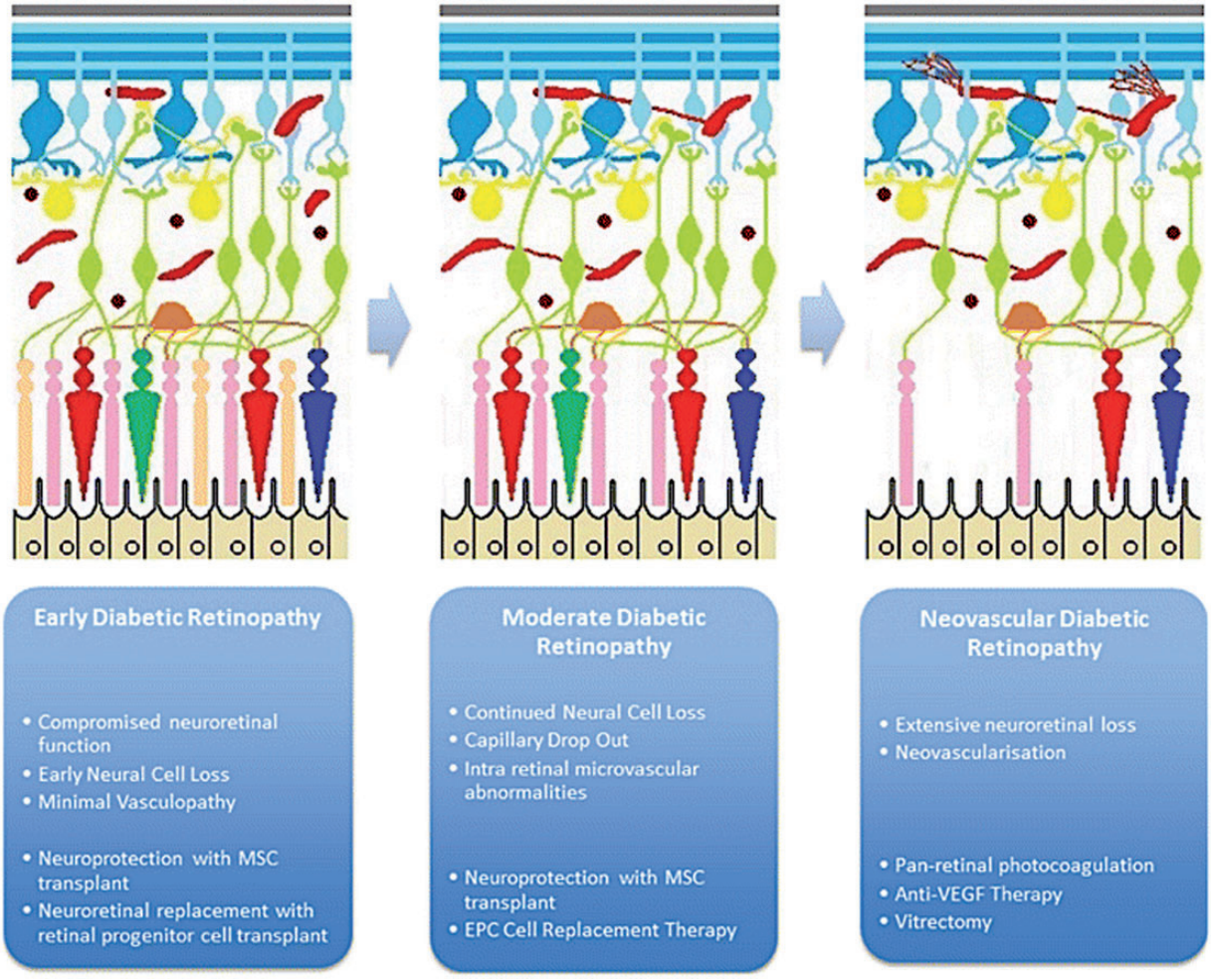

Figure 1 Maximizing the potential efficacy of stem cell therapies will depend on the timing of their use. MSC-derived neuroprotection and neuroretinal cell replacement may help if given early in disease. EPC-derived vascular regeneration may offer benefit right up until end stage disease. Reproduced with permission from Megaw and Dhillon. [23] (A color version of this figure is available in the online journal.)

for age-related macular degeneration (AMD) and retinitis pigmentosa (RP). When injected locally or systemically, engrafted MSCs were reported to provide visual protection and a delay in degeneration.[28] This could be due to stimulation of resident neural progenitors to regenerate neuroretinal tissue,[27] paracrine supply of neuroprotectants[29–31] or their possible differentiation into photoreceptors and retinal pigment epithelium in these disease models.[32–35]

MSCs have a potential as candidates for the treatment of diabetes, although mechanisms of their action in alleviating organ damage (immunomodulatory, neuroprotective, or regenerative) remain disputable. MSCs have immunomodulatory effect as they inhibit differentiation of monocytes into dendritic cells *in vivo*.[36] In addition, MSCs can increase levels of the anti-inflammatory cytokine interleukin (IL)-10 and downregulate levels of the pro-inflammatory IL-12 and interferon-γ.[37–40] The benefit of MSCs for treating diabetes and protecting vascular cells is thought to be due to their production of trophic and immunomodulatory factors.[40,41] This hypothesis was partially corroborated by the finding that normal MSCs transiently alleviated hyperglycemia in NOD mice, whereas NOD MSCs did not.[42] It should be noted that MSCs derived from bone marrow of non-obese diabetic (NOD) mice have decreased capacity for adhesion and migration[43] and reduced ability for retinal differentiation,[44] as well as more pro-inflammatory cytokine profile.

In early diabetes, the loss of pericytes and neuroretinal damage appear to be caused by oxidative stress.[2] MSCs were shown to absorb reactive oxygen species (ROS) via expression of sulfoxide reductase A, which may suggest a mechanism for their neuroprotective effect.[45] When delivered intravitreally, MSCs could protect the neuroretina in degenerative retinal animal models by secreting neurotrophic factors that may prevent apoptosis, stimulate angiogenesis, and promote resident neural progenitors to regenerate neuroretinal tissue.[27,29–31]

It is presently unclear whether dysfunctional tissue recovery is mostly due to trophic factors secreted by MSCs that facilitate survival of degenerating tissue and the endogenous stem cells or to the transdifferentiation of transplanted cells that functionally integrate into the diseased tissue. In this respect, it is worth mentioning that MSCs can self-renew and differentiate into tissues of mesodermal origin.[37,38] However, some data also suggest their ability to turn into other cell types, such as glial and neural cells,[46–49] or pancreatic[50–52] and hepatocyte-like cells.[53,54] Despite considerable debate regarding the ability of MSC-derived cells to express markers' characteristic for fully differentiated ecto- and endodermal lineages, there is growing evidence supporting MSCs potential for generating cell types of multiple lineages suitable for cell therapy in various degenerative and metabolic conditions, including diabetes (reviewed in literatures[32,55,56]). This issue needs further investigation in connection with the retinal changes in degenerative diseases including DR.

Although the exact mechanisms by which MSCs provide neuroprotection for damaged retina still remain somewhat unclear, advantages of MSCs are obvious, and clinical trials may soon be underway examining their effect on visual function in ischemic and diabetic retinopathies. At the same time, their autologous use in diabetic patients may be hampered because of their dysfunctional state. A promising approach for future studies would be normalization of their functions using gene therapy, antioxidants, etc.

Endothelial progenitor cells

For a long time, conventional view was that retinal vasculature develops after birth. This view was contested by a breakthrough study that isolated a population of circulating cells capable to differentiate into endothelial cells and to play a role in adult neovascularization.[57] The presence of circulating cells involved in endothelial repair was supported by the finding that bone marrow transplantation leads to donor-derived endothelial cells in the vessel wall.[58] These rare cells, called endothelial progenitor cells (EPCs), are able to migrate to areas of ischemia and incorporate into sites of active angiogenesis.[57] In fact, retinal ischemia generated by retinal vein occlusion was shown to promote the re-endothelialization of acellular capillaries leading to retinal re-vascularization.[59]

In diabetes, IRMAs contain many endothelial cells and are thought to be a result of the ischemic retina trying to stimulate angiogenesis. While there is a general decline of retinal angiogenesis in diabetes, the slow and gradual character of the disease allows for reparative angiogenesis to occur. If retinal angiogenesis could be targeted successfully, there is a possibility that it could eliminate ischemia that drives DR.

Impaired EPC and HSPC mobilization in diabetes

EPCs represent heterogeneous groups of cells ranging from mostly proangiogenic hematopoietic cells to subsets of hematopoietic stem and progenitor cells (HSPCs).[22,60,61] Circulating EPCs were first identified when human CD34+ cells (a HSPC marker) or mouse flk1+ (endothelial marker also known as VEGF receptor 2, or VEGFR2) cells from the peripheral blood were found to acquire endothelial-like properties *in vitro* and promote neovascularization in response to ischemia.[57] EPCs are usually defined in humans as peripheral mononuclear cells that are positive for the stem cell markers (CD34, VEGFR2 and/or CD133), and can repair damaged vasculature by directly differentiating into endothelial cells (re-endothelialization), or by paracrine actions of EPCs that stimulate resident progenitor cells (neovascularization).[62,63]

Numerous studies showed diabetes-associated changes in EPCs, including a decrease in circulating EPCs,[64] and defects in proliferation and vascular tube formation *in vitro*.[65,66] The number of circulating EPCs is reduced in patients with both types of diabetes,[67,68] which is usually associated with diabetic complications.[69] Additionally, the number of CD34+ cells is decreased in the peripheral blood[70] and their reaction to granulocyte colony stimulating factor (G-CSF) is compromised in diabetic individuals.[71–73] The impaired mobilization of EPCs and HSPCs in diabetes suggests that the bone marrow is also affected by the disease.[74]

Analogous observations were made in diabetic animal studies that revealed decreased numbers of circulating

EPCs and reduced mobilization in response to ischemia[75] or wound injury.[76] Similarly to humans, mice with short (5–8 weeks) duration of streptozotocin (STZ)-induced type I diabetes have impaired HSPC mobilization in response to G-CSF, which is correlated with an increase in HSC numbers in the bone marrow.[71]

With regard to mechanisms of these alterations, it was found that diabetic animals have decreased level of a signaling molecule, C-X-C motif chemokine 12 (CXCL12, also called SDF-1) in local tissues and reduced activation of a mobilization-promoting endothelial nitric oxide synthase (eNOS) pathway in the bone marrow.

Interestingly, mobilization defects depend on microenvironment and take place only in nondiabetic-to-diabetic bone marrow transplantation and not in diabetic-to-nondiabetic transplants. Studies of the stem cell niche revealed that decreased number of osteoblasts and altered innervation are among factors contributing to mobilization defects. There is an extensive network of nerves in the bone marrow, including sympathetic nervous system (SNS) that is needed to mobilize HSPC.[77] Also, inhibition of osteoblast activity and suppression of CXCL12 in the bone by SNS mediate, in part, G-CSF-induced mobilization of HSPCs.[77] Circulating HSPCs were found to display circadian oscillations caused by circadian secretion of noradrenaline by the SNS into the BM, which downregulates CXCL12 via adrenergic receptor on MSCs.[78,79] Involvement of the bone marrow sympathetic nerves in the regulation of EPC release was studied in a rat model of type II diabetes,[80] which revealed a reduction in sympathetic nerve terminals followed by decreased circadian release of EPCs and accumulation of slowly proliferating EPCs in the bone marrow.

These studies strongly implicate dysfunctional stem cell niche in the bone marrow in diabetes and emphasize possible structural and functional changes in the bone marrow induced by diabetes.

EPCs potential for cell replacement

There are two main subtypes of EPCs isolated from the peripheral blood that share certain surface markers, such as CD34, CD131, and KDR.[81,82] One of the subtypes, endothelial colony forming cells (ECFCs), is directly involved in vascular repair by incorporating into mature blood vessels and forming an endothelial layer of the damaged vasculature.[83,84] They can also secrete paracrine factors required for vessel repair.[85] The other subtype, endothelial cell colony forming units (CFU-EPCs) can affect vascular repair only in a paracrine manner, i.e. by releasing growth factors that stimulate resident endothelial progenitors in blood vessel walls.[86]

The use of a mixed cell population to enhance EPCs reparative capacity in a mouse model of limb ischemia yielded some promising results.[87] However, they were not corroborated by subsequent clinical studies[88] suggesting the potential for CFU-EPCs to promote inflammatory response.[84] At the same time, ECFCs became incorporated into retinal vasculature, preventing neovascularization in a mouse model of ischemic retinopathy.[84] Thus, ECFCs appear to be the true EPCs, making their clinical assessment as a homogenous cell type an important issue.

There are some problems with regard to EPCs use for cell replacement. First, it should be noted that intravenously injected EPCs tend to accumulate in liver and spleen.[89] Also, similar to MSCs, a diabetic host environment is a hostile one for EPCs and hinders their migration and adhesion,[90] thus decreasing EPCs reparative potential. Noteworthy, the eye has an advantage, compared to other organs, associated with the opportunity to directly and accurately deliver therapeutic cells to the area of ischemia. This helped to start a clinical trial on the safety of intravitreally injected BM-derived CD34+ cells in hereditary retinal dystrophy.[91]

Diabetic retinopathy, EPC dysfunction and cell therapy

Retinal vasodegeneration is a hallmark of an earlier DR stage, that is, NPDR. In the diabetic environment that is characterized by elevated levels of reactive oxygen species (ROS), vascular progenitors switch to producing pathologic cytokines such as tumor necrosis factor (TNF)-α, IL-8, and to increased expression of pathologic inducible nitric oxide synthase (iNOS) instead of eNOS.[21] As a result, diabetic EPCs have reduced bioavailable NO due to either decreased eNOS activity or increased generation of ROS via upregulated NADPH oxidase.[92] NO-mediated signaling events are also important for the mobilization of EPCs from the bone marrow and in their homing to ischemic regions.[93,94] As shown by the Grant's group, the function of diabetic EPCs can be partially restored by increasing eNOS expression, either by using NO donors, or by reducing NADPH oxidase-dependent ROS production.[95,96] Besides increased ROS and reduced bioavailable NO, several other molecular alterations have been found in dysfunctional diabetic EPCs, including decreased cathepsin L activity[97] and elevated expression of thrombospondin-1.[98]

Regarding the late stage proliferative disease, or PDR, recent studies suggest that the high numbers of bone marrow-derived EPCs constitute a major factor in the development of such serious complications as pathologic neovascularization of ischemic tissues. Although increased numbers of circulating CD34+CD45− endothelial colony-forming cells (ECFCs) were found in PDR patients compared with controls,[99] these cells were defective in their ability to migrate toward SDF-1, incorporate into and form vascular tubes. These data suggest that even though the ECFCs from PDR patients are mobilized into the circulation, they are unable to properly migrate and repair damaged vascular endothelium.[99]

Despite these caveats, cell-based therapy may still represent an effective alternative strategy for the current approaches to the treatment of end-stage DR and other ischemic retinopathies. Cell therapies are designed to target early and intermediate stages of vasodegeneration to promote vascular repair, reverse ischemia, reduce hypoxic or inflammatory signaling, and prevent progression to the late and sight-threatening DR stages.[21] This strategy may prove to be successful if autologous progenitors could be modified to function properly. In recent years,

a number of approaches have been developed to reverse EPC defects in diabetic patients, including improvement of EPC mobilization and homing with G-CSF[100] and SDF-1,[101,102] or use of an NO donor to alleviate SDF-1-mediated migration defects.[95] Further, some evidence suggests that diabetic EPC dysfunction can be improved or corrected by treatment with peroxisome proliferator-activated receptor (PPAR)-δ and -γ agonists GW501516,[103] rosiglitazone,[104] or atorvastatin.[105] Additionally, it was found that the levels of transforming growth factor (TGF)-β1 were significantly increased both in the EPCs (Figure 2) and in the serum of type 2 diabetic patients.[106] TGF-β1 inhibition in CD34+ cells increased cell survival, NO release, and *in vivo* vascular reparative ability (Figure 2), suggesting that this approach could be used for improving the vasoreparative potential of dysfunctional diabetic CD34+ cells for autologous therapy.[106]

Another potential approach for fighting pathological neovascularization at the late proliferative stage of DR may be based on inhibiting protein kinase CK2 that is involved in retinal angiogenesis.[107,108] CK2 inhibitors prevented recruitment of EPCs (Sca-1+/c-kit+ BM-derived HSC) to areas of retinal neovascularization in mouse oxygen-induced retinopathy (OIR) model.[109]

ASC and iPSC

Adipose stem cells (ASCs) are another class of progenitor cells that share characteristics of both MSCs and EPCs. They can be relatively easily harvested by liposuction, isolated from stromal-vascular fraction of fat, and expanded *in vitro* to promote angiogenesis.[110] CD34+ cells isolated from the adipose tissue prevent endothelial apoptosis and stabilize vasculature,[111] and are believed to originate from resident pericytes.[112]

Intravitreally injected ASCs incorporate into retinal vasculature, acquire pericyte position, and prevent retinal endothelial apoptosis and capillary dropout by about 50% and 80%, as was shown in OIR mouse model and Akimba diabetic mice, respectively.[113] Interestingly, similar to native retinal pericytes, the pericyte phenotype of ASCs can be enhanced by TGF-β1 treatment making such ASCs more suitable for cell therapy.[113] Moreover, ASCs intravitreal inoculation into type I diabetic athymic nude rat led to improvement of electroretinogram, thus also providing neuroprotection.[114]

Pluripotent stem cells (PSC) represented until recent discovery of induced PSC (iPSC) mostly by embryonic stem cells (ESC) are able to differentiate into any cell type of all three main lineages. ESC-derived retinal progenitor cells showed their ability to integrate and differentiate into functional photoreceptors, as evidenced by a significant improvement of vision in mouse models of retinal degeneration,[115,116] although it is unclear whether neuroretinal replacement is likely to be successful in diabetes. Much promise for treatment of retinal pigment epithelium (RPE) dystrophies, such as age-related macular degeneration, is associated with ESC-derived RPE[117] that are currently being tested in clinical trials.[118] In diabetes, tight junctions of RPE are compromised leading to breakdown of RPE barrier at late stage hypoxia,[119,120] which suggests that RPE replacement might be also important for managing DR.

Latest advancements in reprogramming adult somatic cells into iPSCs may allow developing a promising strategy for DR treatment. Recently, vascular progenitors have been generated from iPSC derived from CD34+ cord blood cells.[121] Those iPSCs were stimulated to become CD31+/CD146+ vascular progenitors by treatment with high levels of VEGF.[122] Interestingly, engraftment of vascular progenitors generated from iPSCs may occur in different positions in the capillary depending on the way of their delivery. CD31+/CD146+ vascular progenitors injected into the vitreous of NOD/SCID mice that had endured ischemic injury to retina resulting in acellular capillaries, migrated to the abluminal pericyte location of the acellular capillaries. When the cells were administered intravenously, they were incorporated into a lumenal position, assuming their role as endothelial cells.[123] The use of iPSCs made from cord blood CD34+ cells presents a feasible approach to regenerate acellular capillaries. As during reprogramming, iPSC may shed some of their epigenetic changes that are the basis of diabetic metabolic memory, their use for generation of relatively normal autologous vascular progenitors may constitute a viable strategy for an auxiliary DR treatment.

Corneal stem cells changes in diabetes

If one considers diabetic eye disease at large, stem cells could contribute to future treatments not only for retinal vasculature, but also for diabetic corneal alterations known as diabetic keratopathy. The severity of these alterations, e.g. of neuropathy, correlates with the severity of retinopathy.[124] We have recently documented a significant decrease in the expression of a number of putative stem cell markers in the corneolimbal epithelial stem cell compartment.[125] As corneal epithelium is renewed by limbal stem cells, this might explain clinically observed delays in diabetic wound healing, for example, after epithelial debridement for vitrectomy[126] or refractive surgery.[127] Adenoviral gene therapy with overexpression of c-met proto-oncogene and/or silencing of matrix metalloproteinase-10 and cathepsin F normalized epithelial wound healing and stem cell marker expression in human organ-cultured diabetic corneas.[125,128] Importantly, gene therapy of the limbal stem cell niche only produced the same normalization of stem cell marker expression and wound healing.[129] In the future, gene therapy or replacement of ailing stem cells with cultured normal cells including those made from iPSC[130] could become viable options for alleviating diabetic corneal disease.

In summary, stem cells may offer new ways of retarding progression or alleviating symptoms of DR. Currently, their use is considered for earlier stages of DR, before the onset of PDR. Some of these cells secreting special growth factors could serve for neuroprotection in the diabetic retina. Endothelial progenitor cells could be used for preventing and/or repairing capillary closure and reduce pericyte dropout. New strategies to normalize functions of diabetic progenitors offer ways to use them for autologous therapy. The emerging ESC and iPSC technologies may also help

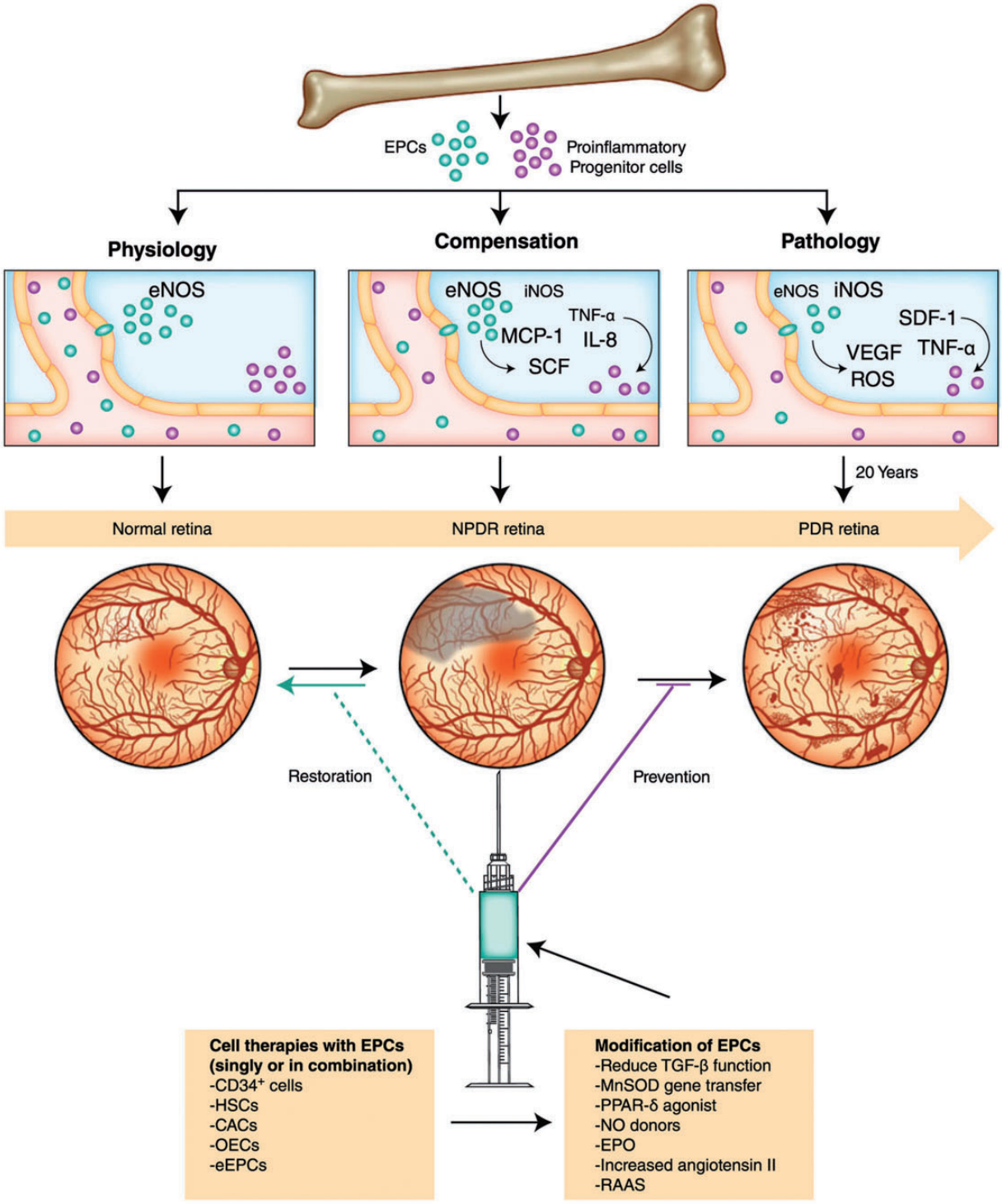

Figure 2 Diabetic dysfunction in the BM mobilization of stem/progenitor cells and paracrine regulation of ischemic vascular repair. In normal conditions, factors released by ischemic/injured tissue cause mobilization of BM cells. In diabetes, there is reduced mobilization of BM cells into circulation. Cell therapy in diabetic retinopathy would ideally restore perfusion to areas of the retina that have undergone vasodegeneration associated with NPDR and would prevent the development of advanced disease, PDR.
BM: bone marrow; CACs: circulating angiogenic cells; eEPCs: early endothelial progenitor cells; eNOS: endothelial nitric oxide synthase; EPCs: endothelial progenitor cells; EPO: erythropoietin; HSCs: hematopoietic stem cells; IL: interleukin; iNOS: inducible nitric oxide synthase; MCP-1: monocyte chemoattractant protein-1; MnSOD: manganese superoxide dismutase; NO: nitric oxide; NPDR: nonproliferative diabetic retinopathy; OECs: outgrowth endothelial cells; PDR: proliferative diabetic retinopathy; PPAR-δ: peroxisome proliferator-activated receptor-δ; RAAS: renin-angiotensin-aldosterone system; ROS: reactive oxygen species; SCF: stem cell factor; SDF-1: stromal cell–derived factor-1; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor. Reproduced with permission from Shaw et al.[21] (A color version of this figure is available in the online journal.)

generate bankable and renewable sources of stem cells capable upon proper differentiation to enhance cellular regeneration in the diabetic retina.

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DECLARATION OF CONFLICTING INTERESTS

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